

**REMARKS**

Claims 12-14, 17 and 18 are amended. Claims 15, 31, 33 and 34 are cancelled. Claims 22-30 are withdrawn from consideration. Claims 12-14, 16-21, 32, 35 and 36 remain active and under consideration.

At the outset, Applicants wish to thank Examiner Marvich for the recent helpful and courteous discussion conducted with their U.S. representative, Mr. William Beaumont. The claims amended herewith and associated remarks are in accordance with that discussion, and are responsive to the Advisory Action of March 24, 2010.

**Claim 36**

Claim 36 is not an ‘added new claim’. This claim corresponds to claim 4 of the PCT application, and to claim 15 of the Preliminary Amendment of this U.S. application. During prosecution, claim 15 was replaced by claim 36. Hence, claim 36 does not present new issues for consideration.

Further, claim 36 is readily distinguishable over the art of record. For example, and in contrast, Poquet et al (Journal of Bacteriology, 1998) merely describe a construct which contains a part of the sequence coding for ZitS. This reference clearly does not read on claim 36 which clearly excludes expression cassettes containing any part of the sequence encoding ZitS. MPEP 2173.05 (i) explicitly sets forth the acceptability of negative limitations in complying with 35 USC 112, second paragraph. Moreover, in this case, the negative limitation “ wherein the

expression cassette does not contain any part of the sequence encoding the *L. lactis* ZitS protein”

provides a clear distinction over Poquet et al as well as the two other references noted in

Advisory Action.

#### Claims 17 and 18

Claims 17 and 18 are amended pursuant to the recent discussion with Examiner Marvich. Hence, the previous objections to these claims is deemed moot.

#### Sequence Listing For AAK06214

As noted previously, the protein sequence deposited under GenBank accession number AAK06214 was available in 2001. See the “Sequence Revision History” from NCBI (of record) which shows that protein AAK06214 was available as of February 9, 2001. The corresponding nucleotide sequence was also available then because the genome of *Lactococcus lactis* strain IL 1403 was also sequenced in 2001. See Bolotin et al., “The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. *Lactis* IL1403”, *Genome Res.*, 2001 May 11(5):731-753. Further, the strain IL1403 is laboratory strain commonly used and widely

accessible.

Thus, Applicants again assert that one skilled in the art would have no difficulty in obtaining the polynucleotide sequence encoding the protein AAK06214, or in reproducing it by synthesis or by screening a DNA library of *Lactococcus lactis* strain IL1403 with probes and/or primers derived from sequence data available in GenBank database. Finally, after having obtained this sequence, the artisan would have no difficulty in incorporating the sequence into an expression cassette according to the claimed invention.

However, Applicants provide herewith a compliant sequence listing and CRF version as requested by the examiner. Further, attached Annex 1 shows the different update dates for sequence AAK06214. May 14, 2001 update ( last update before the priority and filing dates of the application). Annex 2 shows the amino acid sequence of locus AAK06214 that was available at the time of filing. The February 26, 2010 update (Annex 3) gives the same sequence as may 14, 2001 update. See Annex 4.

Accordingly, in view of all of the above, it is believed that this application is now in condition for allowance. Favorable consideration to this effect is earnestly solicited.

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'William E. Beaumont', with a long horizontal flourish extending to the right.

William E. Beaumont  
Reg. No. 30,996  
Juneau Partners, PLLC  
Customer No.: 50438

ANNEX 1



## Sequence Revision History

My NCBI [2]  
[Sign In] [Register]
[PubMed](#) [Nucleotide](#) [Protein](#) [Genome](#) [Structure](#) [PMC](#) [Taxonomy](#) [OMIM](#) [Books](#)

Find (Accessions, GI numbers or Fasta style SeqIds)

AAK06214

Go

Clear

[About Entrez](#)

Show difference between I and II as

[GenBank/GenPept](#)

Entrez

Search for Genes

Entrez Gene provides  
gene-specific data for  
multiple taxa[Help](#) | [FAQ](#)Batch Entrez: Upload a  
file of GI or accession  
numbers to retrieve  
protein or nucleotide  
sequences[Check sequence  
revision history](#)[How to create WWW  
links to Entrez](#)[LinkOut](#)

Related resources

BLAST  
Reference sequence project  
Entrez Gene  
Clusters of orthologous  
groups  
Protein reviews on the web

## Revision history for AAK06214

GI	Version	Update Date	Status	I	II
12725171	1	Feb 26 2010 1:42 PM	Live	⊗	○
12725171	1	Feb 26 2009 8:26 AM	Dead	○	⊗
12725171	1	Jun 2 2004 12:03 PM	Dead	○	○
12725171	1	May 14 2001 12:46 PM	Dead	○	○
12725171	1	Feb 9 2001 4:25 PM	Dead	○	○

Accession AAK06214 was first seen at NCBI on Feb 9 2001 4:25 PM[Disclaimer](#) | [Write to the Help Desk](#)[NCBI](#) | [NLM](#) | [NIH](#)

Last update: Thu, 17 Dec 2009 Rev. 178872

ANNEX 2

My NCBI [2]  
[Sign In] [Register]

PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMM Books

Search Protein for [ ] Go Clear

Display GenPept Show 5 Send to

Range: from begin to end Features: ☒ CDD + Refresh

1: AAK06214. Reports zinc transport tr...[gi:12725171]

BLink, Related  
sequences,  
Identical proteins,  
LinksFeatures Sequence

LOCUS AAK06214 145 aa linear BCT 14-MAY-2001  
DEFINITION zinc transport transcriptional regulator [Lactococcus lactis subsp. lactis].  
ACCESSION AAK06214  
VERSION AAK06214.1 GI:12725171  
DBSOURCE locus AE006439 accession AE006439.1  
KEYWORDS .  
SOURCE Lactococcus lactis subsp. lactis  
ORGANISM Lactococcus lactis subsp. lactis  
Bacteria; Firmicutes; Lactobacillales; Streptococcaceae; Lactococcus.  
REFERENCE 1 (residues 1 to 145)  
AUTHORS Bolotin,A., Wincker,P., Mauger,S., Jaillon,O., Malarme,K., Weissenbach,J., Ehrlich,S.D. and Sorokin,A.  
TITLE The complete genome sequence of the lactic acid bacterium Lactococcus lactis ssp. lactis IL1403  
JOURNAL Genome Res. 11 (5), 731-753 (2001)  
PUBMED 11337471  
REFERENCE 2 (residues 1 to 145)  
AUTHORS Bolotin,A., Wincker,P., Mauger,S., Jaillon,O., Malarme,K., Weissenbach,J., Ehrlich,S.D. and Sorokin,A.  
TITLE Direct Submission  
JOURNAL Submitted (09-JAN-2001) INRA, Genetique Microbienne, Domaine de Vilvert, Jouy en Josas 78352, France  
COMMENT Method: conceptual translation supplied by author.  
FEATURES  
source 1..145  
/organism="Lactococcus lactis subsp. lactis"  
/strain="IL1403"  
/sub\_species="lactis"  
/db\_xref="taxon:1360"  
Protein 1..145  
/product="zinc transport transcriptional regulator"  
Region 22..142  
/region\_name="MarR"  
/note="Transcriptional regulators [Transcription]; COG1846"  
/db\_xref="CDD:32031"  
Region 35..130  
/region\_name="WHTH\_GntR"  
/note="Winged helix-turn-helix (WHTH) DNA-binding domain of the GntR family of transcriptional regulators; c100088"  
/db\_xref="CDD:173998"  
CDS 1..145  
/gene="zitR"  
/gene\_synonym="L168265"  
/coded\_by="complement(AE006439.1:9974..10411)"  
/note="EVIDENCE BY HOMOLOGY BIO09.08 MarR-family regulators. belongs to the MarR family. 48% identical to AdcR repressor of adc locus, affecting competence in Streptococcus pneumoniae."  
/transl\_table=11

ANNEX 3



Protein

My NCBI  
[Sign In] [Register]

PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMIM Books

Search Protein for

Go

Clear

Display GenPept

Show 5

Send to

Range: from begin

to end

Features: ☒ CDD

Refresh

BLink, Related

sequences,

Identical proteins,

Links

1: [AAK06214](#). Reports zinc transport tr...[gi:12725171]Features Sequence

LOCUS AAK06214 145 aa linear BCT 26-FEB-2009  
 DEFINITION zinc transport transcriptional regulator [Lactococcus lactis subsp. lactis IL1403].

ACCESSION AAK06214  
 VERSION AAK06214.1 GI:12725171  
 DBLINK Project:72  
 DBSOURCE accession AE005176.1

KEYWORDS  
 SOURCE Lactococcus lactis subsp. lactis IL1403  
 ORGANISM Lactococcus lactis subsp. lactis IL1403  
 Bacteria; Firmicutes; Lactobacillales; Streptococcace  
 Lactococcus.

REFERENCE 1 (residues 1 to 145)  
 AUTHORS Bolotin, A., Wincker, P., Mauger, S., Jaillon, O., Malarma  
 Weissenbach, J., Ehrlich, S.D. and Sorokin, A.

TITLE The complete genome sequence of the lactic acid bacterium  
 Lactococcus lactis ssp. lactis IL1403

JOURNAL Genome Res. 11 (5), 731-753 (2001)  
 PUBMED 11337471

REFERENCE 2 (residues 1 to 145)  
 AUTHORS Bolotin, A., Wincker, P., Mauger, S., Jaillon, O., Malarma, K.,  
 Weissenbach, J., Ehrlich, S.D. and Sorokin, A.

TITLE Direct Submission  
 JOURNAL Submitted (09-JAN-2001) INRA, Genetique Microbienne, Domaine de  
 Vilvert, Jouy en Josas 78352, France

COMMENT Method: conceptual translation supplied by author.

FEATURES Location/Qualifiers

source

1..145  
 /organism="Lactococcus lactis subsp. lactis IL1403"  
 /strain="IL1403"  
 /sub\_species="lactis"  
 /db\_xref="taxon:272623"

Protein

1..145  
 /product="zinc transport transcriptional regulator"

Region

22..142  
 /region\_name="MarR"  
 /note="Transcriptional regulators [Transcription];  
 COG1846"  
 /db\_xref="CDD:32031"

Region

35..130  
 /region\_name="WHTH\_GntR"  
 /note="Winged helix-turn-helix (WHTH) DNA-binding domain  
 of the GntR family of transcriptional regulators; c100088"  
 /db\_xref="CDD:173998"

CDS

1..145  
 /gene="zitR"  
 /locus\_tag="L168265"  
 /coded\_by="complement(AE005176.1:2184820..2185257)"  
 /note="EVIDENCE BY HOMOLOGY BIO09.08 MarR-family  
 regulators. belongs to the MarR family. 48% identical to  
 AdcR repressor of adc locus, affecting competence in

en cliquant

sur

26/02/2010!

Streptococcus pneumoniae."

/transl\_table=11

ORIGIN

```
( 1 mslanqidqf lgtimqfaen kheillgkce sdvklstqge hilmllaeqi stnakiaekl
  61 kispavtka lkkqlqegeli kssratnder vvlwslteka vpvakehath hektlstyqe
  121 lgnkftdeeq eviskflsal teefq
//
```

[Disclaimer](#) | [Write to the Help Desk](#)[NCBI](#) | [NLM](#) | [NIH](#)

Last update: Fri, 09 Apr 2010 Rev. 188411



Annex 4

aak06214 : Sequence Revision History v3.0 :: NCBI/NLM/NIH - Windows Internet Explorer

http://www.ncbi.nlm.nih.gov/sviewer/girevhist.cgi

Live Search

Fichier Edition Affichage Favoris Outils ?

Favoris Galerie de composan... Sites suggérés

aak06214 : Sequence Revision History v3.0 :...

Page Sécurité Outils

Sequence Revision History

My NCBI Sign In Register

Nucleotide Protein Genome Structure PMC Taxonomy OMIM Books

ns, GI numbers or Fasta style SeqIds

aak06214

Go Clear

Show difference in FASTA format Show

GI	Version	Update Date
12725171	1	Feb 26 2010 1:42 PM
12725171	1	May 14 2001 12:46 PM

>gi|12725171|gb|AAK06214.1|AE006439\_11 zinc transport transcriptional regulator [Lactococcus lactis subsp. lactis I11403]  
>gi|12725171|gb|AAK06214.1|AE006439\_11 zinc transport transcriptional regulator [Lactococcus lactis subsp. lactis]  
MSLANQIDQFLGTIMQFAENKHEILGKCSIDVKLTSTQEHILMLLAEQISTNAKIAEKLKISPAAVTKA  
LKKLQEQELIKSSRATNDERVVVLSLIEKAVFVAKZHATHHEKTLSTYQELGNKFTDEEQEVISKFLSAL  
TEEFQ

Terminé

Internet 100%